FILE 'REGISTRY' ENTERED AT 10:35:43 ON 02 APR 2004

=> S 11 ALPHA HYDROXYLASE/CN
L1 0 11 ALPHA HYDROXYLASE/CN

=> S ALPHA HYDROXYLASE/CN
L2 0 ALPHA HYDROXYLASE/CN

FILE 'CAPLUS' ENTERED AT 10:37:44 ON 02 APR 2004

=> S ASPERGILLUS;S OCHRACEUS;S 11 ALPHA HYDROXYLASE
43748 ASPERGILLUS
545 ASPERGILLI
105 ASPERGILLIS
L3 43843 ASPERGILLUS
(ASPERGILLUS OR ASPERGILLI OR ASPERGILLIS)

L4 1079 OCHRACEUS

827339 11
1467124 ALPHA
2491 ALPHAS
1467222 ALPHA
(ALPHA OR ALPHAS)
37736 HYDROXYLASE
2730 HYDROXYLASES
38241 HYDROXYLASE
(HYDROXYLASE OR HYDROXYLASES)
L5 62 11 ALPHA HYDROXYLASE
(11(W)ALPHA(W)HYDROXYLASE)

=> S L5 AND L3
L6 21 L5 AND L3

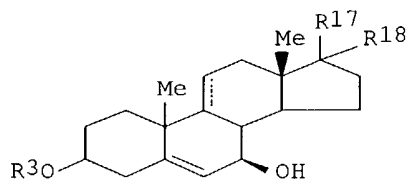
=> S L6 AND L4
L7 14 L6 AND L4

=> S L6 NOT L7
L8 7 L6 NOT L7

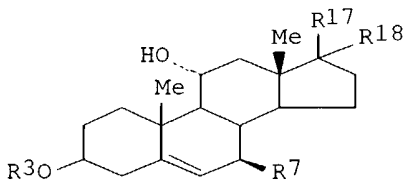
=> D 1-14 L7 CBIB ABS;D L8 1-7 CBIB ABS

L7 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
2004:142848 Document No. 140:199497 Process for preparing 5-androsten-3β-ol steroid intermediates. White, Michael J.; Wuts, Peter Guillaume Marie; Beck, Doris (USA). U.S. Pat. Appl. Publ. US 2004034215 A1 20040219, 21 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-392955 20030321. PRIORITY: US 2002-PV403990 20020816; US 2002-PV415293 20021001.

GI



I



II

AB The present invention discloses a process for the transformation of 5-androsten-3β-ol-17-one to 5-androsten-3β-ol steroid intermediates such as I [R3 = H, COR4; R4 = H, alkyl;

R17R18 = O, lactone; dashed bond = single bond or double bond], and II [R7 = H, OH]. Thus, bioconversion of 5-androsten-3β-ol-17-one (III) to 5-androsten-3β,7β-diol-17-one (IV) was performed using a submerged culture of Diplodia gossypina ATCC 20571. IV was subsequently converted to 5-androsten-3β,7β,11α-triol-17-one (V) using a submerged culture of **Aspergillus ochraceus** ATCC 18500. V can also be obtained from III using a submerged culture of Absidia coerulea ATCC 6647.

L7 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
2002:449846 Document No. 137:29820 Cloning, characterization and use of steroid **11α-hydroxylase** and cytochrome P 450 oxidoreductase from **Aspergillus ochraceus**.
Bolton, Suzanne; Clayton, Robert; Easton, Alan; Engel, Leslie; Messing, Dean (Pharmacia Corporation, USA). PCT Int. Appl. WO 2002046386 A2 20020613, 181 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US51070 20011026. PRIORITY: US 2000-PV244300 20001030.

AB The present invention relates to a novel cytochrome P 450-like enzyme (**Aspergillus ochraceus 11α-hydroxylase**) and an oxidoreductase (**Aspergillus ochraceus** oxidoreductase) isolated from cDNA library generated from the mRNA of **Aspergillus ochraceus** spores. When the cDNA encoding the **11α-hydroxylase** was co-expressed in Spodoptera frugiperda (Sf-9) insect cells with the cDNA encoding human oxidoreductase as an electron donor, it successfully catalyzed the conversion of the steroid substrate 4-androstene-3,17-dione (AD) to 11-α-hydroxy-AD as determined by HPLC anal. The invention also relates to nucleic acid mols. associated with or derived from these cDNAs including complements, homologues and fragments thereof, and methods of using these nucleic acid mols., to generate, for example, polypeptides and fragments thereof. The invention also relates to the generation of antibodies that recognizes the A. **ochraceus 11-. alpha.-hydroxylase** and oxidoreductase and methods of using these antibodies to detect the presence of these native and recombinant polypeptides within unmodified and transformed host cells, resp. The invention also provides methods of expressing the **Aspergillus 11-α-hydroxylase** gene sep., or in combination with human or **Aspergillus** oxidoreductase, in heterologous host cells, to facilitate the bioconversion of steroid substances to their 11-α-hydroxy-counterparts.

L7 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
1999:765135 Document No. 132:34821 Bioconversion of progesterone by the activated immobilized conidia of **Aspergillus ochraceus**
TS. Dutta, Tapan K.; Samanta, Timir B. (Marine Biology Institute, Kamaishi City, 026, Japan). Current Microbiology, 39(6), 309-312 (English) 1999. CODEN: CUMIDD. ISSN: 0343-8651. Publisher: Springer-Verlag New York Inc..

AB Progesterone was transformed to its 11α-hydroxy derivative (100% e.e) by the activated immobilized conidia of **Aspergillus ochraceus** TS. The immobilized preparation retained 79% of free conidial activity. The immobilized conidia, activated by nutrients, exhibited an increase in 11α-hydroxylation, and it was free of the side product 6β, 11α-dihydroxy progesterone. The half life and turnover of immobilized and activated immobilized conidia were 14 and 12 days and 187 and 416 μmoles of the product/g of conidia resp. The pH and temperature profiles of the free conidia remained unaltered after immobilization and activation. Some germination of conidia inside the matrix owing to incubation with nutrients was detected by SEM.

L7 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
1988:18229 Document No. 108:18229 Characterization of progesterone **11α-hydroxylase** of **Aspergillus**

ochraceus TS: a cytochrome P-450 linked monooxygenase. Samanta, Timir B.; Ghosh, Dipak K. (Dep. Microbiol., Bose Inst., Calcutta, 700 009, India). Journal of Steroid Biochemistry, 28(3), 327-32 (English) 1987. CODEN: JSTBBK. ISSN: 0022-4731.

AB The monooxygenase of A. **ochraceus** TS capable of 11α-hydroxylation of progesterone has been resolved into 3 components characterized as cytochrome P 450, NADPH-cytochrome P 450-reductase, and phosphatidylcholine. The **11.alpha .-hydroxylase** was NADPH dependent, and hydroxylation was enhanced by a NADPH-regenerating system. This fungal monooxygenase has many features in common with mammalian liver microsomes. The effects of mammalian cytochrome P 450 inducers were tested for induction of **11α-hydroxylase** in A. **ochraceus** TS. The reductase was partially purified.

L7 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1987:437996 Document No. 107:37996 Solvent damage during free cell catalysis and its avoidance: studies of 11α-hydroxylation of progesterone by **Aspergillus ochraceus**. Ceen, E. G.; Herrmann, J. P. R.; Dunnill, P. (Dep. Chem. Biochem. Eng., Univ. Coll. London, London, WC1E 7JE, UK). Enzyme and Microbial Technology, 9(6), 365-8 (English) 1987. CODEN: EMTED2. ISSN: 0141-0229.

AB The solvent tolerance of the progesterone **11α- hydroxylase** system of A. **ochraceus** was defined and, given its limited extent for conventional organic solvents, a number of natural oils were examined They were superior and represent an interesting solvent class for organic reactants of partial polarity. The study emphasizes that solvents for the products of biocatalytic action on organic reactants must often be partially polar and must not interact strongly with cellular lipids.

L7 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1987:405713 Document No. 107:5713 Solvent damage during immobilized cell catalysis and its avoidance: studies of 11α-hydroxylation of progesterone by **Aspergillus ochraceus**. Ceen, E. G.; Herrmann, J. P. R.; Dunnill, P. (Dep. Chem. Biochem. Eng., Univ. Coll., London, WC1E 7JE, UK). Applied Microbiology and Biotechnology, 25(6), 491-4 (English) 1987. CODEN: AMBIDG. ISSN: 0175-7598.

AB The tolerance towards conventional organic solvents of the progesterone **11α-hydroxylase** system in alginate-immobilized A. **ochraceus** was examined Though greater than that for the enzyme system in free cells it is still too limited for practical use. Substitution of natural oils gave a more stable catalyst system. The activity vs. a free cell catalyst was not attractive in short term use, but may be over the longer term.

L7 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1985:2383 Document No. 102:2383 Studies on the microsomal 11α-hydroxylation of progesterone in **Aspergillus ochraceus**: characterization of the hydroxylase system. Madyastha, K. M.; Jayanthi, C. R.; Madyastha, P. (Dep. Org. Chem., Indian Inst. Sci., Bangalore, 560 012, India). Proceedings - Indian Academy of Sciences, Chemical Sciences, 93(7), 1191-203 (English) 1984. CODEN: PIAADM. ISSN: 0253-4134.

AB From the induced vegetative cell cultures of A. **ochraceus**, a procedure for the preparation of cell-free extract with high **11. alpha.-hydroxylase** activity (I) was developed. To obtain optimal I, EDTA (10 mM), glycerol (10%), and dithiothreitol (5 mM) were required in the grinding medium. Although the optimum pH for the grinding medium was 8.3, the hydroxylase has a pH optimum of 7.7. Microsomes (2 mg) isolated from the crude cell-free extract, hydroxylated progesterone in high yields (85-90% in 30 min) in the presence of NADPH and O2. The apparent Km for NADPH and progesterone were 0.052 mM and 0.625 mM, resp. The involvement of cytochrome P 450 system in the hydroxylation reaction was established by various inhibition studies. I was inhibited by metyrapone, CO, SKF-525A, and p-chloromercuribenzoate. The presence of high levels of NADPH-cytochrome c reductase in the microsomal fraction and the strong inhibition of the hydroxylase system by cytochrome c indicated that the reductase could be a component of the hydroxylase system. Progesterone induced I significantly, whereas deoxycorticosterone and phenobarbital failed to bring about induction. However, deoxycorticosterone acted as a good substrate for I.

The membrane-bound hydroxylase was solubilized using various ionic and non-ionic detergents. Solubilized membrane fraction contained considerable levels of cytochrome P 450 and NADPH-cytochrome c reductase, besides I.

L7 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1984:152928 Document No. 100:152928 Studies on the microsomal 11α-hydroxylation of progesterone in **Aspergillus ochraceus**: isolation, characterization, and solubilization of the hydroxylase system. Madyastha, K. M.; Jayanthi, C. R.; Madyastha, P.; Sumathi, D. (Dep. Org. Chem., Indian Inst. Sci., Bangalore, 560 012, India). Canadian Journal of Biochemistry and Cell Biology, 62(2-3), 100-7 (English) 1984. CODEN: CJBBDU. ISSN: 0714-7511.

AB A suitable procedure for the preparation of cell-free extract with high progesterone **11α-hydroxylase** (I) activity from the induced vegetative cell cultures of A. **ochraceus** was developed. The presence of EDTA, glycerol, and dithiothreitol in the grinding medium was necessary for optimal I activity. Although the pH optimum of I was 7.7, the ideal pH for preparing the cell-free extract from mycelium was 8.3. Microsomes (2.0 mg) isolated from the cell-free extract hydroxylated progesterone in very high yields (85-90% in 30 min) in the presence of NADPH and O. Cytosolic involvement in the hydroxylation reaction was not noticed. The apparent Km values for NADPH and progesterone were 0.052 and 0.625 mM, resp. I was inhibited by metyrapone, CO, SKF-525A, and p-chloromercuribenzoate indicating the involvement of the cytochrome P 450 system in the reaction. Inhibition of I by cytochrome c and the presence of significant levels of NADPH-cytochrome c reductase in the microsomal fraction suggested that the reductase could be 1 of the components of the I system. Membrane-bound I was solubilized using Na cholate. Solubilized membrane fraction contained considerable levels of cytochrome P 450 and NADPH-cytochrome c reductase, in addition to I activity.

L7 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1982:487554 Document No. 97:87554 Microsomal 11α-hydroxylation of progesterone in **Aspergillus ochraceus**: part I: characterization of the hydroxylase system. Jayanthi, C. R.; Madyastha, Prema; Madyastha, K. Madhava (Dep. Org. Chem., Indian Inst. Sci., Bangalore, 560 012, India). Biochemical and Biophysical Research Communications, 106(4), 1262-8 (English) 1982. CODEN: BBRCA9. ISSN: 0006-291X.

AB Microsomes (105,000 g sediment prepared from induced cells of A. **ochraceus** hydroxylated progesterone to 11α- hydroxyprogesterone (11α-OHP) in high yields (85-90% in 30 min) in the presence of NADPH and O. The pH optimum for the hydroxylase was 7.7. However, for the isolation of active microsomes, grinding of the mycelium should be carried out at pH 8.3. Metyrapone, CO, SKF-525A, p-chloromercuribenzoate, and N-methylmaleimide inhibited the hydroxylase activity, indicating the involvement of the cytochrome P-450 system. The inhibition of the hydroxylase by cytochrome c and the presence of high levels of NADPH-cytochrome c reductase in induced microsomes suggest that the reductase could be one of the components in the hydroxylase system.

L7 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1982:99899 Document No. 96:99899 11α-Hydroxylation of progesterone by cell-free preparation of **Aspergillus ochraceus** TS. Ghosh, Dipak; Samanta, Timir B. (Dep. Microbiol., Bose Inst., Calcutta, 700009, India). Journal of Steroid Biochemistry, 14(10), 1063-7 (English) 1981. CODEN: JSTBBK. ISSN: 0022-4731.

AB Studies of the hydroxylation of progesterone at C-11 by a cell-free preparation of A. **ochraceus** TS indicated that the hydroxylating enzyme (**11α-hydroxylase**) is inducible in character and located in postmitochondrial supernatant. The reaction in vitro was exhibited in the presence of $NaIO_4$, indicating the hemoprotein nature of the enzyme. Hydroxylation was stimulated by CN^- and inhibited by metyrapone, suggesting that it may be mediated by cytochrome P-450.

L7 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1970:529540 Document No. 73:129540 Interactions of steroids and fungi. III.

11α-Hydroxylation and degradation of progesterone-4-14C by a cell-free preparation from **Aspergillus ochraceus**. Tan, Liat; Falardeau, Pierre (Dep. Nucl. Med. Radiobiol., Centre Hosp. Univ., Sherbrooke, QC, Can.). Journal of Steroid Biochemistry, 1(3), 221-7 (English) 1970. CODEN: JSTBBK. ISSN: 0022-4731.

AB A cell-free preparation of a 20-keto steroid **11α- hydroxylase** and 17(20)-lyase was obtained from A. **ochraceus** NRRL 405 by rupture of the mycelia in phosphate buffer, pH 7.2, containing EDTA, followed by centrifugation at 43,000 g for 45 min. Hydroxylase and lyase activities were measured independently by reverse isotope dilution and by gas chromatog. with progesterone-4-14C as substrate. The formation of radioactive 11α-hydroxyprogesterone and 11α-hydroxytestosterone was demonstrated, and their radiochem. purity verified by dilution with authentic carrier compds. and crystallization to constant sp. activity.

L7 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1970:107006 Document No. 72:107006 Microbial hydroxylations. V. IIα-hydroxylation of progesterone by cell-free preparations of **Aspergillus ochraceus**. Shibahara, Masayoshi; Moody, James A.; Smith, Leland Leroy (Med. Br., Univ. of Texas, Galveston, TX, USA). Biochimica et Biophysica Acta, 202(1), 172-9 (English) 1970. CODEN: BBACAQ. ISSN: 0006-3002.

AB The 11α-hydroxylation of progesterone and 19-nortestosterone by cell-free enzyme systems made from homogenates of vegetative cell cultures of A. **ochraceus** NRRL 405 is described. The **11. alpha.-hydroxylase** is inducible and remains in the supernatant after centrifugation at 24000 + g. The **11. alpha.-hydroxylase** induced by progesterone or by 19-nor-testosterone is free from the associated 6β-hydroxylase or 17β-hydroxy steroid dehydrogenase activities of vegetative cell cultures of A. **ochraceus** NRRL 405, but induction with 11α-hydroxypro-gesterone yields an enzyme preparation with both 6β- and 11β-hydrox-ylase activities. Although complete conversion of progesterone to 11α-hydroxyprogesterone was accomplished with the cell-free prepns., only 14% conversion of 19-nortestosterone to 11α-hydroxy-19-nortestosterone was possible.

L7 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1960:7733 Document No. 54:7733 Original Reference No. 54:1659h-i,1660a Production from **Aspergillus ochraceus** of oxidases for the selective oxidation of progesterone at position 11. Dulaney, Eugene L. (Merck & Co., Inc.). US 2905593 19590922 (Unavailable). APPLICATION: US .

AB A. **ochraceus** NRRL strain 260-4718, grown on a medium critical as to Zn++ and Cd++ concns. produces an oxidase which converts progesterone (I) to its 11-hydroxy derivative (II). The medium contains sucrose 5, $NaNO_3$ 0.76, K_2HPO_4 0.1, 0.05% $MgSO_4 \cdot 7H_2O$ 0.05, KCl 0.05, $FeSO_4 \cdot 7H_2O$ 0.001%. To prevent the production of other oxidases, the culture should include 0.001-0.002 γ Zn++/ml. and 0.0008-0.003 mg. Cd++/ml. After 24-48 hrs. incubation, 10 mg. I in 2.5 ml. propylene glycol is added. After some hrs. the runs are extracted with $CHCl_3$, dried, dissolved in MeOH, and subjected to chromatography. The compds. are revealed by ultraviolet light, eluted with MeOH, and their production calculated from their optical ds. determined at 2400 A. A typical experiment yielded 4.1% unchanged I, 76.5% II, and 11.2% 6β, 11α-dihydroxyprogesterone.

L7 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN

1960:7732 Document No. 54:7732 Original Reference No. 54:1659e-h Polyoxygenated steroids. (C I B A Ltd.). GB 816922 19590722 (Unavailable). APPLICATION: GB .

AB Pregnanes are oxygenated in at least two of the three positions: 11, 17, and 21, by the action (in any order and without interruption) of enzymes from aerobic cultures of 2 or 3 microorganisms selected from 2 or more of the following groups: (a) Mucorales, Curvularia, **Aspergillus**, Streptomyces, and Coniothyrium; (b) Trichothecium roseum, Leptosphaeria maculans, Cucurbitaria laburni, Acrospira laevis, Lophotrichus hartinii, Melanospora parasitica, and Thielavia terricola; (c) Ophiobolus herpotrichus and Sclerotinia fructicola. Thus, a 3-day old culture of O. herpotrichus on 50 cc. beer wort at 27° was treated with 15 mg. progesterone (I) in 0.75 cc. Me_2CO, agitated for 3 days, a 3-day culture of Curvularia brachyspora added, the mixture agitated 3 days, the mycelium separated, the filtrate extracted with 3 + 30 cc. EtOAc, the exts. washed, dried,

concentrated and the residue paper chromatographed indicates corticosterone. Similarly, aldosterone, cortisone, hydrocortisone, 17α,21-dihydroxyprogesterone, 1-dehydrocortisone, and 1-dehydrohydrocortisone are prepared from the corresponding 17α,21-unsubstituted compds., and with the addition of Rhizopus nigricans or Cunninghamella blakesleeana, I yields 11α,17α,21- trihydroxyprogesterone or a mixture of cortisone and hydrocortisone, resp. Again, cortexone incubated with C. blakesleeana and T. roseum or with Curvularia lunata and L. maculans yields cortisone and hydrocortisone; 1-dehydrocortexone with C. lunata and L. maculans gives 1-dehydrocortisone and 1-dehydrohydrocortisone.

L8 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
1989:455741 Document No. 111:55741 Bioconversion of progesterone by immobilized **Aspergillus** phoenicis. Park, Hee Eun; Kim, Mal Nam (Dep. Biol., Sang Myung Women's Univ., S. Korea). Misaengmul Hakhoechi, 27(1), 70-6 (Korean) 1989. CODEN: MIHCAR. ISSN: 0440-2413.

AB Progesterone bioconversion by immobilized A. phoenicis was studied. Progesterone was converted to 11α-hydroxyprogesterone and 3 minor byproducts. Whole cells of A. phoenicis were immobilized in Ca alginate, κ-carrageenan, or polyacrylamide. Cells immobilized in Ca alginate gels showed the highest activity for 11α-hydroxylation of progesterone. Further hydroxylation of 11α-hydroxyprogesterone by mycelia immobilized in Ca alginate was greatly reduced. Spores of A. phoenicis which were immobilized with Ca alginate and germinated in situ for 25 h showed higher **11α-hydroxylase** activity than entrapped whole mycelia and they maintained their initial enzyme activity for 8 repeated uses. After 16 reuses, the activity declined by ≥30%. When culture media and Zn2+ were introduced into the reaction media, the activity of the immobilized mycelia which had been decreased by many uses was effectively reactivated.

L8 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
1980:527735 Document No. 93:127735 Salting in progesterone hydroxylases from **Aspergillus** niger 12 Y. Abdel-Fattah, A. F.; Badawi, M. A. (Lab. Microb. Chem., Natl. Res. Cent., Cairo, Egypt). Microbiologia Espanola, 30-31, 121-8 (English) 1978. CODEN: MCESAI. ISSN: 0026-2595.

AB Cell-free progesterone hydroxylases were prepared from A. niger 12 Y by salting-in with (NH4)2SO4 and NaCl solns. An (NH4)2SO4 solution of 5% saturation afforded selective extraction of 11β-hydroxylase from the mycelium. Higher concns. of (NH4)2SO4 provided cell-free prepns. containing **11 α-hydroxylase** and 11β-hydroxylase. The extractability of **11α-hydroxylase**
increased with the increase of (NH4)2SO4 saturation, whereas 11β-hydroxylase showed 2 maximum at 15% and 25% satns. The 21-hydroxylase remained unextractable with (NH4)2SO4 solns. up to 30% saturation On the other hand, 1% NaCl solution afforded selective isolation of **11α-hydroxylase**. Higher concns. of NaCl solution gave similar cell-free prepns. containing **11α- hydroxylase** and 11β-hydroxylase. NaCl was also unsuitable for extracting 21-hydroxylase and this enzyme remained in the mycelium. The mycelial debris remaining after NaCl extraction showed higher activity for 21-hydroxylase than those remaining after (NH4)2SO4 extraction

L8 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
1979:134423 Document No. 90:134423 Effect of some agents on the activity of cell-free progesterone. **11α-Hydroxylase**
and 11β-hydroxylase from **Aspergillus** niger 12Y.
Abdel-Fattah, A. F.; Badawi, M. A. (Lab. Microb. Chem., Natl. Res. Cent., Cairo, Egypt). Zentralblatt fuer Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung 2, Naturwissenschaftliche: Mikrobiologie der Landwirtschaft, der Technologie und des Umweltschutzes, 133(7-8), 733-7 (English) 1978. CODEN: ZBPUDE. ISSN: 0323-6056.

AB The effect of some agents on the activity of cell-free progesterone **11α-hydroxylase** and 11β-hydroxylase from A. niger 12 Y was studied. CaCl2, NaCl, MgSO4, CuSO4, and EDTA inhibited **11α-hydroxylase** and
11β-hydroxylase, whereas HgCl2 inhibited only **11. alpha.-hydroxylase**. Inhibition of both the enzymes was also caused by I2, p-chloromercuribenzoate, iodoacetic acid, maleic acid, and cystine as well as potassium ferricyanide for **11. alpha.-hydroxylase**. Reduced

glutathione and cysteine-HCl activated **11α-hydroxylase** and 11β-hydroxylase. The probability of the presence of reactive SH groups in the active sites of both enzymes was discussed. Urea inhibited both fungal progesterone hydroxylases, probably due to enzyme protein denaturation.

L8 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
1976:55896 Document No. 84:55896 Formation and some factors influencing the activity of progesterone hydroxylases by **Aspergillus** niger. Abdel-Fattah, A. F.; Sallam, L. A. R.; El-Refai, A. H.; Zeinel-Abdin, A. (Lab. Microb. Chem., Natl. Res. Cent., Cairo, Egypt). Acta Biologica Academiae Scientiarum Hungaricae, 26(1-2), 43-9 (English) 1975. CODEN: ABAHAU. ISSN: 0001-5288.

AB The formation of progesterone hydroxylases by **Aspergillus** niger 173 was investigated. The constitution of the fermentation medium influenced both the yield and the type of enzymes catalyzing the transformation of progesterone. The enzyme yield also varied with the pH value at which induction was performed as well as with the buffer used. The transformation activity of progesterone was more pronounced with mycelia induced in citrate-phosphate than in phosphate buffer. The results demonstrated that induction of 6β-hydroxylase was favored at pH values near neutrality whereas that of **11α- hydroxylase** was favored in the presence of citrate ions. The transformation activity of progesterone was optimal at pH 5.0. The action of **11α-hydroxylase** was also optimal at pH 5.0, but other hydroxylases showed pH optima between 2.2 and 4.0. Progesterone concns. >6 mg/50 ml reaction mixture was a limiting factor for the rate of transformation activity.

L8 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
1975:590176 Document No. 83:190176 Preparation of cell-free progesterone hydroxylases from **Aspergillus** niger 12Y. Abdel-Fattah, Ahmed F.; Badawi, Mohamed A. (Lab. Microbiol. Chem., Natl. Res. Cent., Cairo, Egypt). Journal of General and Applied Microbiology, 21(4), 217-23 (English) 1975. CODEN: JGAMA9. ISSN: 0022-1260.

AB Preparation of cell-free progesterone hydroxylases from A. niger 12Y was achieved by extraction of the mycelium with acetate, citrate-phosphate, and phosphate buffer solns. at different pH values. All buffer solns. afforded cell-free prepns. containing **11α-hydroxylase,** 11β-hydroxylase, and 21-hydroxylase. The results indicated that **11α-hydroxylase** and 21-hydroxylase comprised the major and minor components of the enzyme prepns., resp. The extractability and(or) the activity of the hydroxylases decreased and increased with the increase of the pH of acetate and citrate-phosphate buffers used in the mycelium extraction, resp. Citrate-phosphate buffer provided enzyme prepns. which were more active than those of acetate buffer. The latter buffer had a harmful effect on both the isolated hydroxylases and those remaining in the mycelium debris. On the other hand, grinding the mycelium with phosphate buffer at pH 6.24 provided the most active enzyme preparation Homogenization of the mycelium with the last-mentioned buffer solution had an adverse effect on the activity of the resulting cell-free preparation Addition of EDTA to the phosphate buffer of pH 6.24 resulted in enzyme prepns. possessing weak **11. alpha.-hydroxylase** and 21-hydroxylase activities and almost no 11β-hydroxylase activity.

L8 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
1975:589707 Document No. 83:189707 Isolation and paper electrophoresis of **Aspergillus** niger 12Y progesterone hydroxylases. Abdel-Fattah, Ahmed F.; Badawi, Mohamed A. (Lab. Microb. Chem., Natl. Res. Cent., Cairo, Egypt). Journal of General and Applied Microbiology, 21(4), 225-32 (English) 1975. CODEN: JGAMA9. ISSN: 0022-1260.

AB Ammonium sulfate was unsuitable for salting out active fractions of progesterone hydroxylases from the cell-free preparation of A. niger 12Y, whereas EtOH provided ppts. possessing weak activities. Me2CO afforded ppts. possessing moderate activities and the precipitate obtained by treatment with 6 volume of Me2CO showed only 11β-hydroxylase activity. Centrifugation of a buffered cell-free preparation at different velocities provided fractions rich in **11α-hydroxylase** and others rich in 11β-hydroxylase. The supernatant fluid remaining after centrifugation at 18,000 rpm and the sediment obtained at 7,000 rpm showed maximal activities of **11. alpha.-hydroxylase** and 11β-hydroxylase, resp. Paper electrophoresis of the cell-free preparation of progesterone hydroxylases was studied with 26 different

buffer solns. Successful separation of **11α-hydroxylase** and 11β-hydroxylase was achieved with acetate buffer of pH 3.42(0.02M and 0.2M) and pH 4.05(0.02M), as well as with phosphate buffer of pH 5.29 (0.0006M) and pH 8.0 (0.0001M). Acetate buffer of low pH had an inhibitory effect on the electrophoresed 11β-hydroxylase. However, both **11. alpha.-hydroxylase** and 11β-hydroxylase became inactive when electrophoresed with acetate buffer of pH 5.89. In no case 21-hydroxylase appeared on the electropherograms, probably due to its presence as a minor component of the enzyme sample.

L8 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
1975:135426 Document No. 82:135426 Production, induction, and activity of progesterone hydroxylases by **Aspergillus** niger 12Y.
Abdel-Fattah, Ahmed F.; Badawi, Mohamed A. (Lab. Microb. Chem., Natl. Res. Cent., Cairo, Egypt). Journal of General and Applied Microbiology, 20(6), 363-71 (English) 1974. CODEN: JGAMA9. ISSN: 0022-1260.

AB Composition of the fermentation medium influenced both the rate of progesterone hydroxylation and the type of the resulting metabolite by A. niger 12Y. Dimethyl sulfoxide was a limiting factor for progesterone hydroxylation but it did not affect the types of enzymic activities. No extracellular hydroxylases catalyzing the transformation of progesterone were produced. Mycelial sporulation catalyzed the productivity of hydroxylases and(or) the rate of progesterone transformation. Productivity of hydroxylases was more inducive with mycelia grown in shaken than in surface culture. Contrary to 11β-hydroxylase, the inducibility of **11. alpha.-hydroxylase** was unaffected by the pH value at the time of induction. 11α-Hydroxy, 17α-hydroxy, and 21-hydroxy derivs. of progesterone induced the formation of only the corresponding hydroxylases. On the other hand, 11β-hydroxyprogesterone induced both 11β- and 21-hydroxylases. Quantities >3 g of induced moist mycelium and concns. of substrate >10 mg in 70 ml of the reaction mixture were limiting factors for progesterone transformation. The in vivo **11α-hydroxylase** and 11β-hydroxylase were most active at a pH of 4.0-6.0, while 21-hydroxylase showed a maximal activity at pH 4.0.

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=> E BOLTON S/AU
=> S E3,E33
            17 "BOLTON S"/AU
             1 "BOLTON SUZANNE"/AU
L9          18 ("BOLTON S"/AU OR "BOLTON SUZANNE"/AU)

=> E BOLTEN S/AU
=> S E4,E5
             4 "BOLTEN SUZANNE"/AU
             6 "BOLTEN SUZANNE L"/AU
L10         10 ("BOLTEN SUZANNE"/AU OR "BOLTEN SUZANNE L"/AU)

=> E CLAYTON R/AU
=> S E3-E5,E9,E11,E12,E17,E18,E19,E21,E43-E56
            21 "CLAYTON R"/AU
            20 "CLAYTON R A"/AU
             1 "CLAYTON R A S"/AU
             3 "CLAYTON R E"/AU
            13 "CLAYTON R H"/AU
             1 "CLAYTON R J"/AU
           173 "CLAYTON R N"/AU
             3 "CLAYTON R P"/AU
             1 "CLAYTON R PAUL"/AU
             1 "CLAYTON R W"/AU
             4 "CLAYTON ROBERT"/AU
             5 "CLAYTON ROBERT A"/AU
             1 "CLAYTON ROBERT A E"/AU
            20 "CLAYTON ROBERT E"/AU
             4 "CLAYTON ROBERT E JR"/AU
             1 "CLAYTON ROBERT EDMUND"/AU
             4 "CLAYTON ROBERT H"/AU
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             1 "CLAYTON ROBERT J"/AU
             5 "CLAYTON ROBERT L"/AU
             3 "CLAYTON ROBERT L JR"/AU
           164 "CLAYTON ROBERT N"/AU
             2 "CLAYTON ROBERT NORMAN"/AU
             1 "CLAYTON ROBERT PAUL"/AU
             2 "CLAYTON ROBERT W"/AU
L11        454 ("CLAYTON R"/AU OR "CLAYTON R A"/AU OR "CLAYTON R A S"/AU OR
                "CLAYTON R E"/AU OR "CLAYTON R H"/AU OR "CLAYTON R J"/AU OR
                "CLAYTON R N"/AU OR "CLAYTON R P"/AU OR "CLAYTON R PAUL"/AU OR
                "CLAYTON R W"/AU OR "CLAYTON ROBERT"/AU OR "CLAYTON ROBERT A"/AU
                 OR "CLAYTON ROBERT A E"/AU OR "CLAYTON ROBERT E"/AU OR "CLAYTON
                 ROBERT E JR"/AU OR "CLAYTON ROBERT EDMUND"/AU OR "CLAYTON ROBER
               T H"/AU OR "CLAYTON ROBERT J"/AU OR "CLAYTON ROBERT L"/AU OR
                "CLAYTON ROBERT L JR"/AU OR "CLAYTON ROBERT N"/AU OR "CLAYTON
                ROBERT NORMAN"/AU OR "CLAYTON ROBERT PAUL"/AU OR "CLAYTON ROBERT
                 W"/AU)

=> E EASTON A/AU
=> S E3,E6,E8-E10
             6 "EASTON A"/AU
             4 "EASTON A M"/AU
             3 "EASTON ALAN"/AU
            13 "EASTON ALAN M"/AU
            19 "EASTON ALAN MICHAEL"/AU
L12         45 ("EASTON A"/AU OR "EASTON A M"/AU OR "EASTON ALAN"/AU OR "EASTON
                 ALAN M"/AU OR "EASTON ALAN MICHAEL"/AU)

=> E ENGEL L/AU
=> S E3,E7,E27,E28
            35 "ENGEL L"/AU
             1 "ENGEL L C"/AU
             4 "ENGEL LESLIE"/AU
             3 "ENGEL LESLIE C"/AU
L13         43 ("ENGEL L"/AU OR "ENGEL L C"/AU OR "ENGEL LESLIE"/AU OR "ENGEL
               LESLIE C"/AU)

=> E MESSING D/AU
=> S E4
L14          2 "MESSING DEAN"/AU

=> E NG J/AU
=> S E3,E10,E12,E13,E59-E66
            53 "NG J"/AU
            52 "NG J N"/AU
             9 "NG J S"/AU
            28 "NG J S T"/AU
             4 "NG JOHN"/AU
            74 "NG JOHN N"/AU
            28 "NG JOHN S"/AU
             1 "NG JOHN SAU HOI"/AU
             6 "NG JOHNNY S T"/AU
             1 "NG JOHNNY SHING TUNG"/AU
             2 "NG JOHNY S"/AU
             1 "NG JOHNY SAU HOI"/AU
L15        259 ("NG J"/AU OR "NG J N"/AU OR "NG J S"/AU OR "NG J S T"/AU OR
                "NG JOHN"/AU OR "NG JOHN N"/AU OR "NG JOHN S"/AU OR "NG JOHN
               SAU HOI"/AU OR "NG JOHNNY S T"/AU OR "NG JOHNNY SHING TUNG"/AU
               OR "NG JOHNY S"/AU OR "NG JOHNY SAU HOI"/AU)

=> E REITZ B/AU
=> S E3,E4,E8-E10
            24 "REITZ B"/AU
            17 "REITZ B A"/AU
             1 "REITZ BEVERLEY"/AU
             1 "REITZ BEVERLY"/AU
             8 "REITZ BEVERLY A"/AU
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L16          51 ("REITZ B"/AU OR "REITZ B A"/AU OR "REITZ BEVERLEY"/AU OR "REITZ
                BEVERLY"/AU OR "REITZ BEVERLY A"/AU)

=> E WALKER MARK/AU
=> S E3-E17
             60 "WALKER MARK"/AU
             18 "WALKER MARK A"/AU
              1 "WALKER MARK ALEXANDER"/AU
              1 "WALKER MARK ALLEN"/AU
              1 "WALKER MARK ANDREW"/AU
              1 "WALKER MARK ANTHONY"/AU
             32 "WALKER MARK C"/AU
              1 "WALKER MARK CROSSFIELD"/AU
              2 "WALKER MARK D"/AU
              1 "WALKER MARK F"/AU
             45 "WALKER MARK J"/AU
              1 "WALKER MARK JOSEPH"/AU
              1 "WALKER MARK LEROY"/AU
              1 "WALKER MARK M"/AU
              1 "WALKER MARK W"/AU
L17         167 ("WALKER MARK"/AU OR "WALKER MARK A"/AU OR "WALKER MARK ALEXANDE
                R"/AU OR "WALKER MARK ALLEN"/AU OR "WALKER MARK ANDREW"/AU OR
                "WALKER MARK ANTHONY"/AU OR "WALKER MARK C"/AU OR "WALKER MARK
                CROSSFIELD"/AU OR "WALKER MARK D"/AU OR "WALKER MARK F"/AU OR
                "WALKER MARK J"/AU OR "WALKER MARK JOSEPH"/AU OR "WALKER MARK
                LEROY"/AU OR "WALKER MARK M"/AU OR "WALKER MARK W"/AU)

=> E WANG PING/AU
=> S E3
L18         1090 "WANG PING"/AU

=> S L9,L10,L11,L12,L13,L14,L15,L16,L17,L18
L19         2132 (L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR
                L18)
=> S L19 AND L5
L20            1 L19 AND L5

=> D CBIB ABS

L20  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2004 ACS on STN
2002:449846   Document No. 137:29820  Cloning, characterization and use of
      steroid 11α-hydroxylase and cytochrome
      P 450 oxidoreductase from Aspergillus ochraceus.  Bolton, Suzanne
      ; Clayton, Robert; Easton, Alan; Engel, Leslie
      ; Messing, Dean (Pharmacia Corporation, USA).  PCT Int. Appl. WO
      2002046386 A2 20020613, 181 pp.  DESIGNATED STATES: W:  AE, AG, AL, AM,
      AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK,
      DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP,
      KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
      MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR,
      TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
      TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
      GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.
      (English).  CODEN: PIXXD2.  APPLICATION: WO 2001-US51070 20011026.
      PRIORITY: US 2000-PV244300 20001030.
AB    The present invention relates to a novel cytochrome P 450-like enzyme (Aspergillus
      ochraceus 11α-hydroxylase ) and an oxidoreductase (Aspergillus ochraceus oxidoreductase)
      isolated from cDNA library generated from the mRNA of Aspergillus ochraceus spores. When
      the cDNA encoding the 11α-
      hydroxylase was co-expressed in Spodoptera frugiperda (Sf-9) insect cells with the cDNA
      encoding human oxidoreductase as an electron donor, it successfully catalyzed the
      conversion of the steroid substrate 4-androstene-3,17-dione (AD) to 11-α-hydroxy-AD as
      determined by HPLC anal.  The invention also relates to nucleic acid mols. associated with
      or derived from these cDNAs including complements, homologues and fragments thereof, and
      methods of using these nucleic acid mols., to generate, for example, polypeptides and
      fragments thereof.  The invention also relates to the generation of antibodies that
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recognizes the A. ochraceus **11-α-hydroxylase** and oxidoreductase and methods of using these antibodies to detect the presence of these native and recombinant polypeptides within unmodified and transformed host cells, resp. The invention also provides methods of expressing the Aspergillus **11-α-hydroxylase** gene sep., or in combination with human or Aspergillus oxidoreductase, in heterologous host cells, to facilitate the bioconversion of steroid substances to their 11-α-hydroxy-counterparts.

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=> S ALPHA HYDROXYLASE
       1467124 ALPHA
          2491 ALPHAS
       1467222 ALPHA
                  (ALPHA OR ALPHAS)
         37736 HYDROXYLASE
          2730 HYDROXYLASES
         38241 HYDROXYLASE
                  (HYDROXYLASE OR HYDROXYLASES)
L21       4223 ALPHA HYDROXYLASE
                  (ALPHA(W)HYDROXYLASE)

=> S L21 AND L19
L22          1 L21 AND L19

=> S L22 NOT L20
L23          0 L22 NOT L20
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